Chapter 2

Antimicrobial coatings to prevent biofilm formation

2.1 Biofilm formation on biomaterials

A biofilm is formed when microorganisms adhere to a surface, start to grow and encapsulate themselves in a self-produced extracellular matrix. Biofilms have multiple impacts on the Earth's features: they can be essential (in sewerage processing or food digestion), inconvenient (on slippery steps), unpleasant (smelly),[1-4] destructive (within water conduits) or can even be life threatening when occurring in the human body.[5] In modern medicine, especially in the application of biomaterials implants, such as joint prostheses, contact lenses, voice prostheses or indwelling catheters,[6] biofilms can have disastrous consequences,[1-4] particularly when diagnosed too late or for implants difficult to replace.

Biofilm formation starts with initial, reversible bacterial adhesion to a surface. After initial adhesion, bacteria anchor themselves irreversibly to the surface through unfolding of surface appendages or production of a biofilm matrix consisting of extracellular polymeric substances. After irreversible anchoring, bacteria proliferate and accumulate and the protective biofilm is created.[5,7] When a biofilm has matured, two possible mechanisms for the detachment and dispersal of biofilm bacteria exist.[8] One mechanism involves a programmed set of chemical events (quorum sensing) proceeding within the biofilm leading to local hydrolysis. As a result, bacteria detach from the biofilm and turn into a planktonic stage. The second mechanism involves physical detachment by environmental forces. Usually only parts of a biofilm are detached by these forces that are subsequently carried away by fluid flow into the environment and possibly adhere elsewhere to initiate the formation of a new biofilm. In the human body, both mechanisms prevail and can cause a severe local infection or bacteremia.

The biofilm matrix is a slimy polymeric substance that holds the bacterial community together. It is a complex mixture of macromolecules including exo-polysaccharides, proteins, eDNA and humic acids.[9-10] The biofilm matrix, although it is known to contain channels, retards diffusion of antimicrobials, depending on the particular type of drug and biofilm.[8] A decrease in susceptibility to antibiotics up to 500-fold has been reported for bacteria in their biofilm mode of growth compared to planktonic bacteria.[11-13] Also nutrient limitation and the resulting slow-growth or starvation state as existing in a biofilm contribute to the reduced susceptibility of biofilms to antimicrobials.[8] Likewise, the biofilm mode of bacterial growth hinders host immune cells in attacking the embedded bacteria. The resistance of biofilms to antimicrobials and to the host immune system are at the root of many persistent and chronic bacterial infections, making prevention mandatory.[8]

Therefore we here evaluate the literature concerning coatings that resist biofilm formation on surfaces by killing bacteria as soon as they adhere to the coated surface with emphasis to the surfaces of biomaterials implants and devices.

2.2 Prevention of biofilm formation on biomaterials

Nature itself provides remarkable examples of efficiently preventing growth of biofilms onto surfaces of living species. For instance, the skin of whales and dolphins stays clean of biofilms by using both passive (a gel-like surface substance) and active mechanisms (enzymatic digestion of undesired biological materials).[14] Synthetic analogues by combining active and passive approaches have been explored as well, incorporating antimicrobials in an antifouling hydrogel[15] or using zwitterionic materials to switch between antifouling and antimicrobial modes.[16] Physico-chemical surface properties of biomaterials, such as hydrophilicity, smoothness, charge and biocidal activity have a major influence on the formation of a biofilm. Controlling these features can reduce or even prevent initial adhesion of bacteria and as a result discourage biofilm formation. Well-known surface coatings for biomaterials to reduce adhesion are formed by hydrophilic polymer brushes.[17-19] Hydrophilic bacterial repellent polymer brushes create dispersion forces between the dangling polymer chains and bacteria, preventing them from recognizing and binding to the surface and initiating biofilm growth. The concept is similar to that of steric stabilization of colloids where polymer chains grafted on the surface of particles prevent agglomeration. Hydrophobicity, combined with a smooth surface, also influences adhesion of bacteria. Intrinsic hydrophobic polymers, like polyvinylidene fluoride[20] and polydimethylsiloxane,[21] are well known polymers showing little bacterial adhesion. Surface roughness affects biofilm formation substantially. Rough surfaces are more inclined to attract biofilms than smooth ones and also more difficult to clean, since bacteria nest themselves preferentially in grooves. With respect to biofilm formation, it is therefore desirable to make surfaces of biomaterials implants as smooth as possible.[22-23]

2.3 Prevention of biofilm formation using leachables from biomaterials or coatings

Numerous materials comprising leachable antimicrobials, to prevent biofilm formation, have been described.[24-26] The leaching antimicrobials kill or stop growth of bacteria as soon as they approach a surface and therewith prevent the formation of a biofilm. The oldest example of the application of a leachable antimicrobial is probably silver.[27] Already ancient cultures around the world used silver jars to keep water and other liquids free from bacterial contamination. Today there is still a medical interest in silver comprising coatings for antibacterial purposes. Considering the mechanism by which silver ions interfere with bacterial cell surfaces, it is conceivable however that silver has a similarly toxic effect on human cells as on bacteria.[28] Silver ions form complexes with proteins and DNA that are abundantly present in both cell types. This might be a reason why the use of silver comprising coatings *in vivo* has been limited to temporarily indwelling devices. Moreover, there are doubts on the effectiveness of using silver *in vivo*.[29] Besides the possible toxic effects of the leachables, there is one other big disadvantage of leachables: coatings or materials containing leachables sooner or later become exhausted and only work during the first period of their application.

2.4 Prevention of biofilm formation with immobilized antimicrobial coatings on biomaterials

Recently, it has been shown that the above mentioned disadvantages of leachables-based preventive measures can be avoided by immobilizing antimicrobial agents on biomaterials surfaces. Antimicrobials can be anchored onto biomaterials surfaces by covalent bonds. Several tethered antimicrobials have been investigated, like lysozyme,[30] antimicrobial peptides[31] and quaternary ammonium compounds (QUATs).[32] Here we focus on QUATs, as they are synthetically well accessible[32] and used already for a long time in disinfectants.

2.5 Mechanism of QUAT molecules as an antimicrobial

QUATs can be classified into low and high molecular weight compounds. Low molecular weight compounds are generally soluble and active in solution, while high molecular polymeric QUATs are not or less soluble and mostly used in coatings.[32] Low molecular weight, soluble QUATs have been used since the 1930s in clinical, domestic and industrial applications.[33] With only a few reports of bacterial resistance, most notably against Pseudomonas species,[34] no apparent reduction of their efficacy exists due to the development of bacterial resistance. Although most of these compounds have rather simple structures, *i.e.* a positive charge and a hydrophobic tail, they are nevertheless very potent antimicrobials. In spite of their long history, the mechanism by which QUATs operate on the molecular level is not yet known in detail. In one proposed mechanism, the polymeric QUATs penetrate through the peptidoglycan layer into the cytoplasmic membrane, while disordering this membrane (Figure 2.1).[35-36]

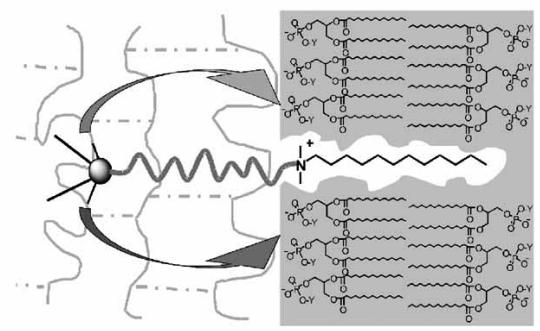

Figure 2.1 Schematic presentation of the disturbance of the cytoplasmic membrane by QUATs in solution. Figure reprinted with permission from Waschinski *et al.*[35]

In this mechanism, QUATs are only effective if they have a hydrophobic tail length, matching the length of the phospholipids of the cytoplasmic membrane. Penetration of QUATs in the phospholipid membrane causes leakage of the cytoplasma, leading to cell death. In a related mechanism, it is proposed that QUATs destabilize the bacterial cell membrane by displacement of cationic ions like magnesium, calcium, sodium and potassium which normally stabilize the membrane (Figure 2.2).[37-40] When positively charged ions enter the cell membrane, other cationic ions have to leave in order to maintain charge neutrality. Divalent calcium ions that are abundantly present in the bacterial cell membrane are not only counter ions of membrane compounds, such

as teichoic acids, but are also responsible for the stability of the membrane network, due to the ionic interactions. Exchanging the divalent calcium ions by monovalent QUATs will accordingly destabilize the cell membrane. In spite of the lack of the details on the molecular level, it is not impossible that both mechanisms simultaneously play a role in the biocidal action of QUATs.

Immobilization of QUATs has also been described as a promising route to obtain a long lasting efficacy.[32,41] However, it is difficult to envisage that the same mechanisms hold for immobilized QUATs as for soluble ones. Recently, a new mechanism was proposed in which the biocidal properties of immobilized QUATs are attributed to stress deformation of the lipid membrane in the target bacteria. This deformation is caused by strong electrostatic interaction forces between immobilized QUATs and negatively charged bacterial cell surfaces,[42] becoming so strong that the rigid bacterial peptidoglycan layer deforms and the cell membrane becomes disrupted to cause leakage of the cytoplasm and bacterial death. This is supported by the fact that the surface charge density of immobilized QUATs has to surpass a threshold of 10^{14} QUATs per cm^2 in order to effective kill adhering bacteria.[40,43]

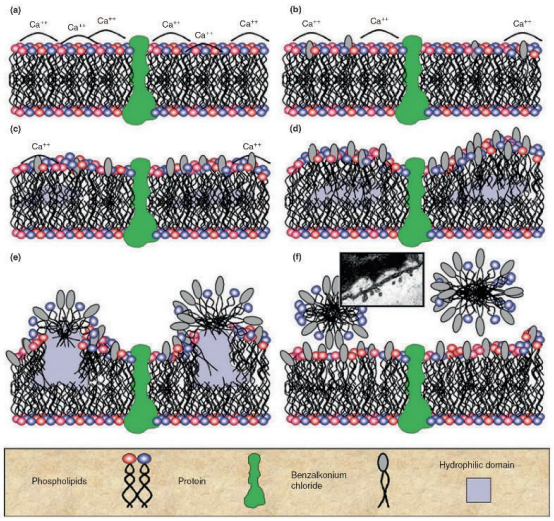

Figure 2.2 Mechanism of cell membrane disturbance by a QUAT, benzalkonium chloride. Sections (a) to (f) show the increasing concentration of the QUATs and a decrease of calcium ions on the membrane. The increasing concentration of QUATs form QUAT/phospholipid micelles leading to lysis of the bacteria. Figure reprinted with permission from Gilbert *et al.*[33]

2.6 Methods for immobilization of antimicrobials to a surface

A universal method to create biomaterials implants and devices with intrinsic antimicrobial functionalities is difficult,[44] because depending on the application, different implant and devices need distinct mechanical and physico-chemical surface characteristics. Immobilizing antimicrobial coatings on existing biomaterials is a relative simple and can be very helpful in preventing biomaterial associated infections. Immobilization of antimicrobial coatings can be achieved by physisorption which relies on polar, electrostatic and hydrophobic interactions and also the relative weak Lifshitz - Van der Waals forces may play a role. However, polymers that are bound by physisorption and not covalently bound to a surface can easily become damaged during handling, washed off from the surface or enzymatically degraded.[45] In contrast, covalent anchored polymers ensure a much greater stability. A number of anchoring methods have been developed to form robust covalent bonds between a polymer and a biomaterial surface. To enable a chemical linkage, the surface should first be furnished with reactive groups like –OH, $–NH_2$ or –COOH. Various methods, including plasma treatments, γ-radiation and wet chemical methods have been explored to modify existing surfaces and to create desired functionalities, including antimicrobial ones. The fixation of pre-synthesized polymers (grafting onto) can be achieved by a reactive polymer group with a functional group on a surface. Another option is to initiate a polymerization from an initiator that is linked to the surface of the biomaterial surface (grafting from). The latter method yields a higher polymer density.[32,46]

2.7 Cationic Polymers

As aforementioned, polymers comprising QUATs are one of the most studied classes of polymers able to kill bacteria. A large variety of QUAT-comprising polymers has been proposed, but most of them are not covalently immobilized.[47] Within the class of the immobilized QUAT-polymers, polyethyleneimines, polyvinylpyridine and polyacrylates are the most important ones and therefore will be briefly reviewed.

2.7.1 Polyvinylpyridine

Alkylated poly(vinylpyridine) (PVP, Figure 2.3) is one of the most thoroughly investigated antimicrobial cationic polymers.[48-49]

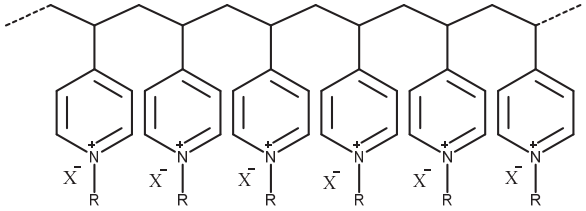

Figure 2.3 Schematic structure of alkylated poly(vinylpyridine). R = alkyl group, X^- = counter ion.

The nitrogen atoms of the pyridine ring have been alkylated with various alkyl halides (RX) to form aromatic QUAT moieties. PVP has been grafted on various surfaces, such as polyethylene terephthalate (PET),[50-51] poly(vinylidene fluoride),[51] cotton,[52] cellulose,[50] glass[53] and

polypropylene.[54] When quaternized PVP coatings, with alkyl moieties ranging from propyl to hexadecyl, were applied on glass, it appeared that the polymers with a hexyl group gave the best results in killing bacteria upon contact.[53] Using various antimicrobial activity tests, like aerosol culturing assays and the shake flask assay (ASTM E2149), most of the PVP based polymers reduced bacterial growth very effectively against a wide range of bacteria (*Escherichia coli, Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* including 4 methicillin-resistant strains and *Streptococcus pneumonia)*.[55] The bacterial killing efficiency was dependent on the charge density of the QUATs on the surface.

2.7.2 Polyethyleneimine

Also polyethylenimine (PEI, Figure 2.4) has been studied extensively to impart antimicrobial properties to a variety of materials. Alkylated PEI has been applied on surfaces by physisorption as well as via chemical attachment. In the latter case, reactive sites on the surface are used to tether the polymer covalently. For example, PEI (M_w = 750 kDa) was tethered to an amino-functional glass surface mediated by 4-bromobutyryl chloride and subsequently quaternized with alkyl bromides.[56] Antimicrobial activity was determined for *S. aureus S. epidermidis*, *P. aeruginosa*, *E. coli*, *Saccharomyces cerevisiae* and *Candida albicans*. An aerosol bacterial assay and shake flask assay demonstrated a reduction of microbial growth of more than 90%. A decrease in the biocidal activity was found when low molecular weight alkylated PEI (< 2 kDa) was used. This was attributed to the inability of the latter compound to penetrate into the cell membrane.[57-58] Glass surfaces coated with N,N-dodecyl, methyl-PEI killed not only human bacterial pathogens, such as *E. coli* and *S. aureus*, but even some influenza viruses were killed with a 100% efficiency.[59-60] Recently it was demonstrated *in vivo* (in sheep), that these coatings (N,N-dodecyl, methyl-PEI) when applied on an orthopedic titanium and stainless steel fracture hardware, completely prevented biofilm formation.[61] An important side effect was, that the coating also supported bone healing. Unfortunately, in most papers, leaching of biocidal compounds from the coated surface cannot be ruled out, as the washing procedures of the coatings are often insufficient.

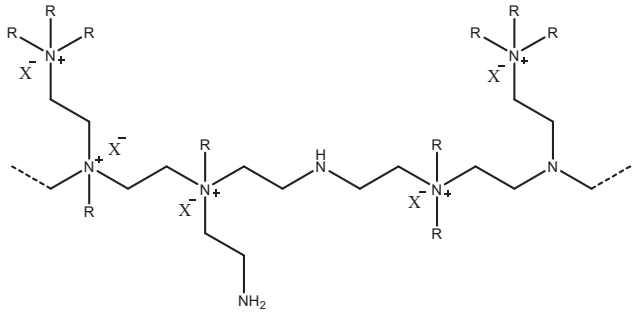

Figure 2.4 Schematic structure of alkylated poly(ethyleneimine). R = alkyl group, X^- = counter ion.

2.7.3 Poly(meth)acrylates

Finally, grafted antibacterial poly(meth)acrylate coatings (Figure 2.5) are also well studied. The (meth)acrylate monomers are mostly polymerized from an immobilized initiator on the surface. Particularly atom transfer radical polymerization is a very useful living polymerization technology to covalently attached poly(meth)acrylates on surfaces.

The (meth)acrylate monomers, for the preparation of biofilm reducing polymers, can contain tertiary amine groups that are subsequently quaternized with alkyl halides. Alternatively, monomers already containing QUAT groups have been directly polymerized as well. QUAT-functionalized polyacrylates were tethered to amino-functional glass surfaces,[62] cellulose[62] and polypropylene.[63] The bacterial contact-killing efficacy of such coatings on glass and cellulose was evaluated using the dynamic shake flask assay (ASTM E2149) against *E. coli* and *Bacillus subtilis* and more than 99.8% of all contacting bacteria were killed. Using the dynamic shake flask assay, poly acrylate treated polypropylene coatings were found to be effective against *E. coli*. The charge density of the QUATs on acrylate treated polypropylene was above the threshold of 10^{14} QUATs per cm^2, which is needed in order to be effective.[40,43] In another study 2-(dimethylamino ethyl)acrylate was polymerized from an immobilized nitroxide-mediated initiator on stainless steel and subsequently quaternized. The coating showed 7 log-units reduction towards *S. aureus* and *E. coli*.[64] Also, series of methacrylate polymers were grafted from silicon and glass surfaces using protected antimicrobial monomers. Upon de-protection, these coatings reportedly killed 100% of *S. aureus* and *E. coli* in less than 5 min, as measured in a JIS Z2801 assay. Interestingly, the killing efficiency did not depend on the thickness of the coatings, *i.e.* the length of polymer chains. Even 3 nm thick coatings were biocidal. This implies that penetration through the peptidoglycan layer (~50 nm) to disturb the cytoplasmic membrane is not the mode of action and points to other mechanisms that may involve excessively high local adhesion forces.[65]

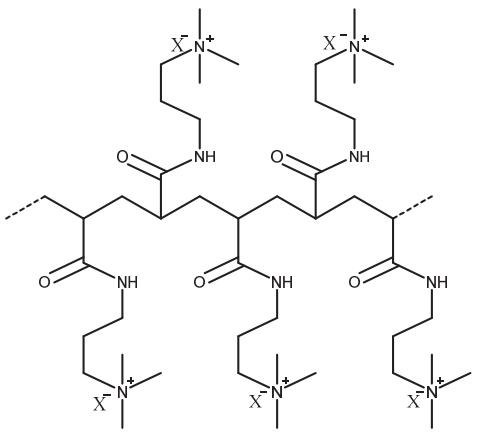

Figure 2.5 Schematic structure of grafted poly (3-methacrylamidopropyl) trimethylammonium chloride. X^- = counter ion.

2.8 Summary

No matter how clean surgical procedures in an operating theater are, it will be very hard to completely prevent bacterial contamination during implant and device surgery due to the ubiquitous presence of bacteria. During implant surgery, bacteria can invade into the wound as well as adhere to the implant or device surface. Bacteria have a general tendency to adhere on surfaces on which they proliferate to their benefit while producing an extra-cellular matrix that protects them against the immune system and antimicrobials. Therefore, there is a need for an interface on biomaterials implants and devices (and surgical instrumentation for that matter) which either repels bacteria or kills bacteria upon contact. Repelling surfaces retard adhesion of bacteria, but currently do not fully prevent it. Biocidal interfaces are therefore a better approach, which can be applied on virtually all biomaterials used for implants or devices.

On one hand, we have soluble QUATs that are well-known, potent antimicrobials and have been used for decades in many applications. A drawback of these soluble compounds in biomaterials coatings is that they will become depleted and eventually contaminate body fluids. Therefore on the other hand, immobilized QUAT coatings have recently attracted much attention, as they do not release QUATs and as a consequence do not cause contamination of the implant or device environment. In the meantime, a variety of polymers comprising QUATs have been studied and a number of them have been immobilized on various biomaterials surfaces. It appeared that such immobilized coatings are effective in bacterial contact killing, provided the charge density on the surface is above a threshold of 10^{14} QUATs per cm^2.

References

[1] Neu, T. R.; De Boer, C. E.; Verkerke, G. J.; Schutte, H. K.; Rakhorst, G.; Van der Mei, H. C.; Busscher, H. J., Biofilm Development in Time on a Silicone Voice Prosthesis — a Case Study. *Microbial Ecology in Health and Disease* **1994,** 7, 27-33.

[2] Everaert, E.; Mahieu, H. F.; Van de Belt-Gritter, B.; Peeters, A.; Verkerke, G. J.; Van der Mei, H. C.; Busscher, H. J., Biofilm Formation in Vivo on Perfluoro-Alkylsiloxane-Modified Voice Prostheses. *Archives of Otolaryngology-Head & Neck Surgery* **1999,** 125, 1329-1332.

[3] Neu, T. R.; Van der Mei, H. C.; Busscher, H. J.; Dijk, F.; Verkerke, G. J., Biodeterioration of Medical-Grade Silicone Rubber Used for Voice Prostheses: A Sem Study. *Biomaterials* **1993,** 14, 459-464.

[4] Elving, G. J.; Van der Mei, H. C.; Busscher, H. J.; Van Weissenbruch, R.; Albers, F., Influence of Different Combinations of Bacteria and Yeasts in Voice Prosthesis Biofilms on Air Flow Resistance. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* **2003,** 83, 45-55.

[5] Jenkinson, H. F.; Lappin-Scott, H. M., Biofilms Adhere to Stay. *Trends in microbiology* **2001,** 9, 9-10.

[6] Bryers, J. D., Biofilms and the Technological Implications of Microbial Cell Adhesion. *Colloids and Surfaces B: Biointerfaces* **1994,** 2, 9-23.

[7] Monroe, D., Looking for Chinks in the Armor of Bacterial Biofilms. *Plos Biology* **2007,** 5, 2458-2461.

[8] Costerton, J. W.; Stewart, P. S.; Greenberg, E. P., Bacterial Biofilms: A Common Cause of Persistent Infections. *Science* **1999,** 284, 1318-1322.

[9] Sutherland, I. W., The Biofilm Matrix – an Immobilized but Dynamic Microbial Environment. *Trends in microbiology* **2001,** 9, 222-227.

[10] Whitchurch, C. B.; Tolker-Nielsen, T.; Ragas, P. C.; Mattick, J. S., Extracellular DNA Required for Bacterial Biofilm Formation. *Science* **2002,** 295, 1487-1487.

[11] Williams, I.; Venables, W. A.; Lloyd, D.; Paul, F.; Critchley, I., The Effects of Adherence to Silicone Surfaces on Antibiotic Susceptibility in *Staphylococcus aureus*. *Microbiology* **1997,** 143, 2407-2413.

[12] Evans, R. C.; Holmes, C. J., Effect of Vancomycin Hydrochloride on *Staphylococcus epidermidis* Biofilm Associated with Silicone Elastomer. *Antimicrobial Agents and Chemotherapy* **1987,** 31, 889-894.

[13] Costerton, J. W.; Lewandowski, Z.; Caldwell, D. E.; Korber, D. R.; Lappin-Scott, H. M., Microbial Biofilms. *Annual Review of Microbiology* **1995,** 49, 711-745.

[14] Baum, C.; Fleischer, L. G.; Roessner, D.; Meyer, W.; Siebers, D., A Covalently Cross-Linked Gel Derived from the Epidermis of the Pilot Whale *Globicephala Melas*. *Biorheology* **2002,** 39, 703-717.

[15] Cheng, G.; Xue, H.; Li, G.; Jiang, S., Integrated Antimicrobial and Nonfouling Hydrogels to Inhibit the Growth of Planktonic Bacterial Cells and Keep the Surface Clean. *Langmuir* **2010,** 26, 10425-10428.

[16] Jiang, S.; Cao, Z., Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications. *Advanced Materials* **2010,** 22, 920-932.

[17] Fundeanu, I.; Van der Mei, H. C.; Schouten, A. J.; Busscher, H. J., Polyacrylamide Brush Coatings Preventing Microbial Adhesion to Silicone Rubber. *Colloids and Surfaces B-Biointerfaces* **2008,** 64, 297-301.

[18] Zhang, Y.; Kohler, N.; Zhang, M., Surface Modification of Superparamagnetic Magnetite Nanoparticles and Their Intracellular Uptake. *Biomaterials* **2002,** 23, 1553-1561.

[19] Saldarriaga Fernández, I. C.; Busscher, H. J.; Metzger, S. W.; Grainger, D. W.; Van der Mei, H. C., Competitive Time- and Density-Dependent Adhesion of Staphylococci and Osteoblasts on Crosslinked Poly(Ethylene Glycol)-Based Polymer Coatings in Co-Culture Flow Chambers. *Biomaterials* **2011,** 32, 979-984.

[20] Brady, R., Clean Hulls without Poisons: Devising and Testing Nontoxic Marine Coatings. *Journal of Coatings Technology* **2000,** 72, 45-56.

[21] Krishnan, S.; Weinman, C. J.; Ober, C. K., Advances in Polymers for Anti-Biofouling Surfaces. *Journal of Materials Chemistry* **2008,** 18, 3405-3413.

[22] Subramani, K.; Jung, R. E.; Molenberg, A.; Hammerle, C., Biofilm on Dental Implants: A Review of the Literature. *The International Journal of Oral & Maxillofacial Implants* **2008,** 24, 616-626.

[23] Quirynen, M.; Bollen, C., The Influence of Surface Roughness and Surface-Free Energy on Supra-and Subgingival Plaque Formation in Man. *Journal of Clinical Periodontology* **1995,** 22, 1-14.

[24] Siepmann, F.; Siepmann, J.; Walther, M.; MacRae, R. J.; Bodmeier, R., Polymer Blends for Controlled Release Coatings. *Journal of Controlled Release* **2008,** 125, 1-15.

[25] Kazemzadeh-Narbat, M.; Lai, B. F. L.; Ding, C.; Kizhakkedathu, J. N.; Hancock, R. E. W.; Wang, R., Multilayered Coating on Titanium for Controlled Release of Antimicrobial Peptides for the Prevention of Implant-Associated Infections. *Biomaterials* **2013,** 34, 5969-5977.

[26] Zhuk, I.; Jariwala, F.; Attygalle, A. B.; Wu, Y.; Libera, M. R.; Sukhishvili, S. A., Self-Defensive Layer-by-Layer Films with Bacteria-Triggered Antibiotic Release. *ACS Nano* **2014,** 8, 7733-7745.

[27] Maillard, J.-Y.; Hartemann, P., Silver as an Antimicrobial: Facts and Gaps in Knowledge. *Critical Reviews in Microbiology* **2013,** 39, 373-383.

[28] Greulich, C.; Braun, D.; Peetsch, A.; Diendorf, J.; Siebers, B.; Epple, M.; Köller, M., The Toxic Effect of Silver Ions and Silver Nanoparticles Towards Bacteria and Human Cells Occurs in the Same Concentration Range. *RSC Advances* **2012,** 2, 6981-6987.

[29] Pickard, R.; Lam, T.; MacLennan, G.; Starr, K.; Kilonzo, M.; McPherson, G.; Gillies, K.; McDonald, A.; Walton, K.; Buckley, B.; Glazener, C.; Boachie, C.; Burr, J.; Norrie, J.; Vale, L.; Grant, A.; N'Dow, J., Antimicrobial Catheters for Reduction of Symptomatic Urinary Tract Infection in Adults Requiring Short-Term Catheterisation in Hospital: A Multicentre Randomised Controlled Trial. *The Lancet* **2012,** 380, 1927-1935.

[30] Muszanska, A. K.; Busscher, H. J.; Herrmann, A.; Van der Mei, H. C.; Norde, W., Pluronic–Lysozyme Conjugates as Anti-Adhesive and Antibacterial Bifunctional Polymers for Surface Coating. *Biomaterials* **2011,** 32, 6333-6341.

[31] Muszanska, A. K.; Rochford, E. T. J.; Gruszka, A.; Bastian, A. A.; Busscher, H. J.; Norde, W.; Van der Mei, H. C.; Herrmann, A., Antiadhesive Polymer Brush Coating Functionalized with Antimicrobial and Rgd Peptides to Reduce Biofilm Formation and Enhance Tissue Integration. *Biomacromolecules* **2014,** 15, 2019-2026.

[32] Kugel, A.; Stafslien, S.; Chisholm, B. J., Antimicrobial Coatings Produced by "Tethering" Biocides to the Coating Matrix: A Comprehensive Review. *Progress in Organic Coatings* **2011,** 72, 222-252.

[33] Gilbert, P.; Moore, L. E., Cationic Antiseptics: Diversity of Action under a Common Epithet. *Journal of Applied Microbiology* **2005,** 99, 703-715.

[34] Langsrud, S.; Sundheim, G.; Borgmann-Strahsen, R., Intrinsic and Acquired Resistance to Quaternary Ammonium Compounds in Food-Related Pseudomonas Spp. *Journal of Applied Microbiology* **2003,** 95, 874-882.

[35] Waschinski, C. J.; Herdes, V.; Schueler, F.; Tiller, J. C., Influence of Satellite Groups on Telechelic Antimicrobial Functions of Polyoxazolines. *Macromolecular Bioscience* **2005,** 5, 149-156.

[36] Tossi, A.; Sandri, L.; Giangaspero, A., Amphipathic, A-Helical Antimicrobial Peptides. *Peptide Science* **2000,** 55, 4-30.

[37] Lenoir, S.; Pagnoulle, C.; Galleni, M.; Compère, P.; Jérôme, R.; Detrembleur, C., Polyolefin Matrixes with Permanent Antibacterial Activity: Preparation, Antibacterial Activity, and Action Mode of the Active Species. *Biomacromolecules* **2006,** 7, 2291-2296.

[38] Chen, C. Z.; Cooper, S. L., Interactions between Dendrimer Biocides and Bacterial Membranes. *Biomaterials* **2002,** 23, 3359-3368.

[39] Norris, V.; Chen, M.; Goldberg, M.; Voskuil, J.; McGurk, G.; Holland, B., Calcium in Bacteria: A Solution to Which Problem? *Molecular Microbiology* **1991,** 5, 775-778.

[40] Kügler, R.; Bouloussa, O.; Rondelez, F., Evidence of a Charge-Density Threshold for Optimum Efficiency of Biocidal Cationic Surfaces. *Microbiology* **2005,** 151, 1341-1348.

[41] Waschinski, C. J.; Zimmermann, J.; Salz, U.; Hutzler, R.; Sadowski, G.; Tiller, J. C., Design of Contact-Active Antimicrobial Acrylate-Based Materials Using Biocidal Macromers. *Advanced Materials* **2008,** 20, 104-108.

[42] Asri, L. A. T. W.; Crismaru, M.; Roest, S.; Chen, Y.; Ivashenko, O.; Rudolf, P.; Tiller, J. C.; Van der Mei, H. C.; Loontjens, T. J. A.; Busscher, H. J., A Shape- Adaptive, Antibacterial- Coating of Immobilized Quaternary-Ammonium Compounds Tethered on Hyperbranched Polyurea and Its Mechanism of Action. *Advanced Functional Materials* **2014,** 24, 346-355.

[43] Murata, H.; Koepsel, R. R.; Matyjaszewski, K.; Russell, A. J., Permanent, Non-Leaching Antibacterial Surfaces—2: How High Density Cationic Surfaces Kill Bacterial Cells. *Biomaterials* **2007,** 28, 4870-4879.

[44] Yue, J.; Zhao, P.; Gerasimov, J. Y.; Van de Lagemaat, M.; Grotenhuis, A.; Rustema-Abbing, M.; Van der Mei, H. C.; Busscher, H. J.; Herrmann, A.; Ren, Y., 3d-Printable Antimicrobial Composite Resins. *Advanced Functional Materials* **2015,** 25, 6756-6767.

[45] Roosjen, A.; de Vries, J.; Van der Mei, H. C.; Norde, W.; Busscher, H. J., Stability and Effectiveness against Bacterial Adhesion of Poly(Ethylene Oxide) Coatings in Biological Fluids. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **2005,** 73B, 347-354.

[46] Dalsin, J. L.; Messersmith, P. B., Bioinspired Antifouling Polymers. *Materials Today* **2005,** 8, 38-46.

[47] Xue, Y.; Xiao, H.; Zhang, Y., Antimicrobial Polymeric Materials with Quaternary Ammonium and Phosphonium Salts. *International journal of molecular sciences* **2015,** 16, 3626-3655.

[48] Sellenet, P. H.; Allison, B.; Applegate, B. M.; Youngblood, J. P., Synergistic Activity of Hydrophilic Modification in Antibiotic Polymers. *Biomacromolecules* **2007,** 8, 19-23.

[49] Allison, B. C.; Applegate, B. M.; Youngblood, J. P., Hemocompatibility of Hydrophilic Antimicrobial Copolymers of Alkylated 4-Vinylpyridine. *Biomacromolecules* **2007,** 8, 2995-2999.

[50] Cen, L.; Neoh, K. G.; Kang, E. T., Surface Functionalization Technique for Conferring Antibacterial Properties to Polymeric and Cellulosic Surfaces. *Langmuir* **2003,** 19, 10295-10303.

[51] Cen, L.; Neoh, K. G.; Ying, L.; Kang, E. T., Surface Modification of Polymeric Films and Membranes to Achieve Antibacterial Properties. *Surface and Interface Analysis* **2004,** 36, 716-719.

[52] Cen, L.; Neoh, K. G.; Kang, E. T., Antibacterial Activity of Cloth Functionalized with N-Alkylated Poly(4-Vinylpyridine). *Journal of Biomedical Materials Research Part A* **2004,** 71A, 70-80.
[53] Tiller, J. C.; Liao, C.-J.; Lewis, K.; Klibanov, A. M., Designing Surfaces That Kill Bacteria on Contact. *Proceedings of the National Academy of Sciences of the United States of America* **2001,** 98, 5981-5985.
[54] Badyal, J. P. S.; Teare, D. O. H.; Schofield, W. C. E. A Method for Producing a Nitrogen Functionalised Surface. WO/2006/111711, **2006**.
[55] Tiller, J. C.; Lee, S. B.; Lewis, K.; Klibanov, A. M., Polymer Surfaces Derivatized with Poly(Vinyl-N-Hexylpyridinium) Kill Airborne and Waterborne Bacteria. *Biotechnology and bioengineering* **2002,** 79, 465-471.
[56] Lin, J.; Qiu, S.; Lewis, K.; Klibanov, A. M., Bactericidal Properties of Flat Surfaces and Nanoparticles Derivatized with Alkylated Polyethylenimines. *Biotechnology progress* **2002,** 18, 1082-1086.
[57] Milović, N. M.; Wang, J.; Lewis, K.; Klibanov, A. M., Immobilized N-Alkylated Polyethylenimine Avidly Kills Bacteria by Rupturing Cell Membranes with No Resistance Developed. *Biotechnology and bioengineering* **2005,** 90, 715-722.
[58] Lin, J.; Qiu, S.; Lewis, K.; Klibanov, A. M., Mechanism of Bactericidal and Fungicidal Activities of Textiles Covalently Modified with Alkylated Polyethylenimine. *Biotechnology and bioengineering* **2003,** 83, 168-172.
[59] Haldar, J.; An, D.; de Cienfuegos, L. A.; Chen, J.; Klibanov, A. M., Polymeric Coatings That Inactivate Both Influenza Virus and Pathogenic Bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **2006,** 103, 17667-17671.
[60] Haldar, J.; Weight, A. K.; Klibanov, A. M., Preparation, Application and Testing of Permanent Antibacterial and Antiviral Coatings. *Nature Protocols* **2007,** 2, 2412-2417.
[61] Schaer, T. P.; Stewart, S.; Hsu, B. B.; Klibanov, A. M., Hydrophobic Polycationic Coatings That Inhibit Biofilms and Support Bone Healing During Infection. *Biomaterials* **2012,** 33, 1245-1254.
[62] Lee, S. B.; Koepsel, R. R.; Morley, S. W.; Matyjaszewski, K.; Sun, Y.; Russell, A. J., Permanent, Nonleaching Antibacterial Surfaces. 1. Synthesis by Atom Transfer Radical Polymerization. *Biomacromolecules* **2004,** 5, 877-882.
[63] Russell, A.; Koepsel, R.; Lee, S.; Matyjaszewski, K. Antimicrobial Surfaces and Methods for Preparing Antimicrobial Surfaces. 2008/0286319, **2008**.
[64] Ignatova, M.; Voccia, S.; Gilbert, B.; Markova, N.; Mercuri, P. S.; Galleni, M.; Sciannamea, V.; Lenoir, S.; Cossement, D.; Gouttebaron, R.; Jerome, R.; Jerome, C., Synthesis of Copolymer Brushes Endowed with Adhesion to Stainless Steel Surfaces and Antibacterial Properties by Controlled Nitroxide-Mediated Radical Polymerization. *Langmuir* **2004,** 20, 10718-10726.
[65] Madkour, A. E.; Dabkowski, J. M.; Nusslein, K.; Tew, G. N., Fast Disinfecting Antimicrobial Surfaces. *Langmuir* **2009,** 25, 1060-7.